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A comparison of the effect of genotype and weather conditions on the nutritional composition of most important commercial nuts

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ABSTRACT

The beneficial effects of nut consumption on human health have been largely confirmed. However, these benefits are variable as a wide range of factors have main influence on the nutritional composition of nuts. This is the case of the specific cultivar considered, as different almond, pistachio and walnut cultivars produce nuts with differentiated composition. Still, there are factors beyond cultivar effect that need to be further analysed. This is the case of weather conditions during nut development. In this study, we evaluate the influence of genotype and the crop year (weather conditions) on nuts health-promoting properties. With that purpose, the nutritional composition of 29 different nut cultivars has been evaluated during two consecutive crop years (2015–2016). The objective of this study was to evaluate in which extend uncontrollable weather conditions during nut development affect the nutritional composition of almonds, pistachios and walnuts. This variability was compared to that resulted from the use of different cultivars. Results show that by using different cultivars, some of the main nutritional parameters of nuts, such as oil content or concentration of linoleic can be controlled. However, for some of the decisive factors on nut nutritional quality, such as protein content, phytosterols concentration and presence of minerals such as iron or sulphur, the weather conditions of the considered crop year are determinant. In this scenario, the health-promoting properties of nuts might be compromised by the climate variability associated to climate change.

1. Introduction

The interest of nut consumption is associated to its health-promoting properties. Nuts consumption improves the flood lipoprotein profile, shows anticancer and antitherogenic effect, contributes to the regulation of immunological activity and inflammatory response, shows high antioxidant capacity, and reduce mediators of chronic diseases such as oxidative stress, visceral adiposity, insulin resistance, endothelial dysfunction or the metabolic syndrome (Abbey et al., 1994; Amaral et al., 2005; Awad and Fink, 2000; De Souza et al., 2017; Gentile et al., 2007; Kodad et al., 2011; Martínez et al., 2006; Robbins et al., 2011; Yang et al., 2009). Moreover, nut consumption is not associated to weight gain, even in large amounts (Hollis and Mattes, 2007; Ibarrola-Jurado et al., 2013; Katz et al., 2012).

Generally, nuts show high lipid content. In almonds, lipids compound 40–67% of dry weight (Yada et al., 2011), with similar values for pistachio, around 50–62% (Catalán et al., 2017). In walnuts, this concentration is even higher, reaching 60–72% of walnut dry weight (Amaral et al., 2003; Kodad et al., 2016). Among lipids, nuts contain high concentration of unsaturated fatty acids, mainly oleic, that constitutes more than 50% of total lipids in almond and pistachio (Catalán et al., 2017; Roncero et al., 2016), and linoleic that exceeds 50% of total lipids in walnuts (Crews et al., 2005; Martínez et al., 2006). Beyond fatty acids, nuts contain interesting concentrations of high quality proteins, as they show high digestibility and high concentration of essential amino acids (Sze-Tao and Sathe, 2000). Nuts also have remarkable concentrations of essential minerals (Roncero et al., 2016), such as magnesium, calcium and potassium, that together prevent bone demineralization, reducing blood pressure and the risk of coronary heart disease (Segura et al., 2006).

Recently, most of the research regarding nuts has focused on minor components due to their influence on nuts nutritional quality. Nuts are an interesting source of vitamin E as they have high content of tocopherols. Total tocopherol concentration ranges from 194 to 297 mg/kg in walnut (Amaral et al., 2005) and 350–550 mg/kg in almonds (Kodad et al., 2014) with average values around 350 mg/kg in pistachios (Ling et al., 2016). Other minor components of interest are phytosterols. They appear in very high concentrations in pistachio oil, up to 271.9 mg/100 g of oil. In almond and walnut oils, phytosterols concentration is lower, around 140–160 mg/100 g of oil (Crews et al., 2005;

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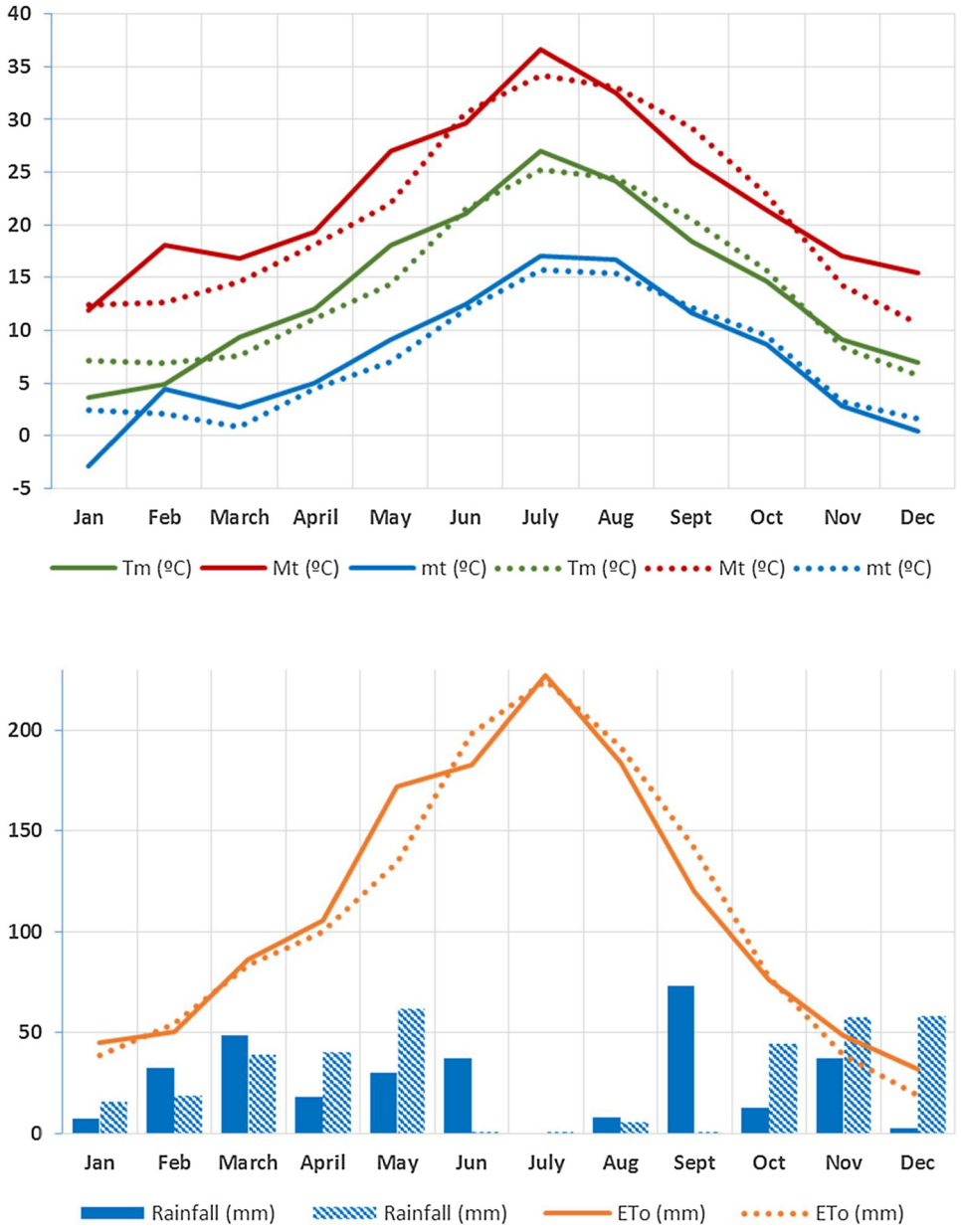

Fig. 1. Weather conditions measured at the crop station during 2015 and 2016 crop years.
Abbreviations: Average monthly temperatures (Tm), average maximum monthly temperatures (Mt), average minimum monthly temperatures (mt) and reference evapotranspiration (ETo). Solid lines and bars show data from 2015 and dotted lines and bars show data from 2016.

Kornsteiner-Krenn et al., 2013).

All the positive effects of nuts on human health are the result of the presence and concentrations of previously mentioned components among others. However, these components vary attending to the nut considered, but also attending to other factors, such as the specific cultivar selected. Almond, pistachio and walnut have been largely spread around the Mediterranean basin and other areas with Mediterranean type climates, such as California, Central Asia and Australia. This expansion has resulted in the development of different cultivars adapted to the environmental conditions of their growing areas or the preferences of local markets. All these cultivars compose a huge genetic resource of plant material that should be studied in order to face the uncertain conditions for the production of nuts due to climate change.

Different pistachio cultivars show differences in the fatty acid profile, triglycerides, phytosterols, amino acids and minerals (Bellomo and Fallico, 2007; Bellomo et al., 2009; Dyszel and Pettit, 1990; Küçüköner and Yurt, 2003; Mahmoodabadi et al., 2012). In almonds, significant differences have been found in the total oil content, fatty acid profile, triglycerides or tocopherols (Kodad et al., 2014, 2011; Roncero et al., 2016; Yada et al., 2011). Similar conclusions have been reported for walnuts (Amaral et al., 2005, 2003; Bada et al., 2010; Crews et al., 2005; Kodad et al., 2016).

Beyond cultivar, weather conditions and cultural practises on the crop field are also important factors to consider, as they all have effects on nut nutritional properties (Carbonell-Barrachina et al., 2015; García-López et al., 1996; Kodad et al., 2014; Sánchez-Bel et al., 2008; Yada et al., 2011; Zhu et al., 2015). Pistachio cultivars grown under different conditions in Tunisia (Chahed et al., 2008; Ghrab et al., 2010), Turkey (Harmankaya et al., 2014; Küçüköner and Yurt, 2003; Seferoglu et al., 2006) or Iran (Kamangar and Farsam, 1977; Mahmoodabadi et al., 2012) show significant differences in their chemical parameters. Similar results have been obtained for walnuts (Amaral et al., 2005; Crews et al., 2005) and almonds (García-López et al., 1996; Maestri

et al., 2015; Roncero et al., 2016; Yada et al., 2011) grown under different conditions.

When different cultivars are grown on different places, the influence of ecological conditions and cultural practises can cause differences in nuts that cannot be attributed to the cultivar itself. For instance, it has been proved the direct effect of watering on the tocopherol content of almonds and walnuts (Amaral et al., 2005; Bada et al., 2010; Crews et al., 2005; Kodad et al., 2016). Most of nut research has evaluated nuts obtained from different cultivars, produced also in different places, with few studies considering different cultivars grown on the same spot under the same weather conditions (Bellomo and Fallico, 2007; Maestri et al., 2015; Tsantili et al., 2011, 2010).

The effect of genotype, crop year and the interaction of both on nutritional composition of nuts has been recently described (Martínez et al., 2006; Rabadán et al., 2018a, b). However, analysis of a bigger picture, including further nutritional analysis and comparison of how these factors affect main nut crops in the world is still needed. These analysis will provide useful information about the nutritional components of almonds, pistachios and walnuts that can be controlled by using specific cultivars and about those that are more dependent on uncontrollable weather conditions on the field crop.

2. Materials and methods

2.1. Plant material

Nuts were collected in the Instituto Técnico Agronómico Provincial of Albacete in the southeast of Spain in 2015 and 2016. Ten almond cultivars (*Antoñeta, Ayles, Belona, Ferraduel, Ferragnes, Guara, Marcona, Penta, Tardona* and *Vairo*), ten pistachio cultivars (*Aegina, Alpina, Avidon, Kerman, Larnaka, Mateur, Napoletana, Ouleimy, Sfax* and *Sirora*) and nine walnut cultivars (*Scharch-Franquette, Hartley, Hugget, Mayette, Mollar de Nerpio, Parisienne, Payne, Pedro* and *Serr*) were considered. One kilogram of kernels were collected from every one of the three trees analysed within each cultivar. Nuts were picked at the most appropriate harvest date for each cultivar.

Although nut production is mainly concentrated in areas with Mediterranean or Mediterranean type climates, differences in water deficit (Zhu et al., 2015) and temperatures (Kodad et al., 2006) should always be considered, as they have major influence in nuts characteristics. As a result, the characterization of genotypes must always be linked to the characteristics of a geographic location. Weather conditions at the experimental plot were kernels were collected are showed in Fig. 1. For the considered years, the precipitation was below the historical average in 2015 and on the average in 2016. Supplemental irrigation was added both years in order to meet crop water requirements. Average temperatures were within the historical records; however, in 2015, temperatures were significantly higher in February, May and December compared to 2016. No fertilizer was added in the two years study.

2.2. Methodology

Nuts were cracked and shelled manually in controlled conditions for immediate drying at room temperature. Oil extraction was carried out using a hydraulic press (MECAMAQ Model DEVF 80, Vila-Sana, Lleida, Spain) at pressure of 150 bar. One kilogram of ground nuts were placed each time on the press. After pressing, oil was centrifuged to remove remaining solids. Oil was stored in dark glass bottles at 5 °C to avoid degradation until analysis (Rabadán et al., 2017a, c).

In order to determine fatty acids composition (%), the methyl-esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 N methanolic potassium hydroxide solution, and analyzed by GC with a Hewlett-Packard chromatograph (HP 6890). A fused silica column (50 m length × 0.25 mm i.d.), coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor), was used. Helium was employed as a carrier gas with a flow through the column of 1 mL min-1. The temperatures of the injector and detector were set at 250 °C with an oven temperature of 210 °C. An injection volume of 1 μL was used (Regulation EEC 2568/91, corresponding to AOCS method Ch 2–91).

The concentration of total polyphenols (ppm) was estimated using the method proposed by Bail et al. (2008) based on the determination of total phenols of oil using the Folin–Ciocalteau colorimetric method. The absorption of the solution was measured on a spectrophotometer Hewlett-Packard 8450 A UV/Vis.

The tocopherol content (mg/Kg) was analysed by HPLC (model 360, Kontron, Eching, Germany) in accordance with the IUPA2432 method (IUPAC, 1947). 1.5 g oil was dissolved in the mobile phase (10 mL) of 0.5% isopropanol in n-hexane. A normal phase column Lichrosphere Si60 (250 mm length x 4.6 mm i.d. and 5 μm particle size; Merck) was used with an injection volume of 20 μL and a flow rate of 1.0 mL/min.

Sterols were determined with a Hewlett-Packard (HP 6890) gas chromatograph with a capillary column (25 m length × 0.25 mm i.d.) coated with SGL-5 (0.25 μm thickness; Sugerlabor). Working conditions were as follows: carrier gas, helium; flow through the column, 1.2 mL min-1; injector temperature, 280 °C; detector temperature, 290 °C; oven temperature, 260 °C; injection volume 1 μL (Regulation EEC 2568/91, corresponding to AOCS method Ch 6–91). Apparent β-sitosterol was calculated as the sum of β-sitosterol, $\Delta^{5,23}$-stigmastadienol, clerosterol, sitostanol, and $\Delta^{5,24}$-stigmastadienol.

Protein content in nuts flour was calculated by multiplying the total nitrogen content, obtained by the Kjeldahl method (FOSS, 2003), by a conversion factor of 5.18 for almond and 5.30 for walnut and pistachio (Greenfield and Southgate, 2003). To determine ash content, flours were ashed at 550 °C to constant weight. Crude fat (ethyl ether extract) was estimated gravimetrically by filter bag technique after petroleum ether extraction of the dried sample in an extraction system Ankom XT10 (ANKOM, 2009). To determine the content of crude fibre, the Weende technique adapted to the filter bag technique was applied. This method determines the organic residue remaining after digestion with solutions of sulfuric acid and sodium hydroxide, using an Ankom 220 fibre analyser (ANKOM, 2008). Total carbohydrate content was calculated by subtracting the sum of the crude protein, total fat, water and ash from the total weight of the flour (Sullivan, 1993). Available carbohydrate content (nitrogen-free) was calculated by subtracting the crude fibre from the total carbohydrate content (González et al., 1987).

Contents of mineral elements in flours were obtained by inductively coupled plasma mass spectrometry (ICP-MS), using Jobin Yvon JY 50-P equipment (Instruments S.A., Jobin Yvon, Longjuemeau, France).

2.3. Statistical analyses

Determinations in this study are means of triplicate measurements from three independent samples for each nut genotype involved in the two-year study. Statistical differences were estimated by ANOVA test at the 5% level ($P \leq 0.05$) of significance. In order to analyze the comparative responses of nut genotypes in both 2015 and 2016 crop years and the possible interaction, data were analyzed by two-way ANOVA. The total variability of each chemical parameter was estimated using the total sum of squares of two-way ANOVA results. Additionally, the variability expressed as percentage of the total sum of squares for genotype, crop year and the interaction between both was calculated. All statistical analysis were carried out using the SPSS programme, release 23.0 for Windows.

3. Results and discussion

The beneficial effects of nuts consumption have been largely reported (De Souza et al., 2017). Nuts are mainly compound by lipids; above 50% of nut dry weight, proteins and carbohydrates (Catalán et al., 2017; Rabadán et al., 2017b; Yada et al., 2011). The total variance observed for each nutritional component and the factor that

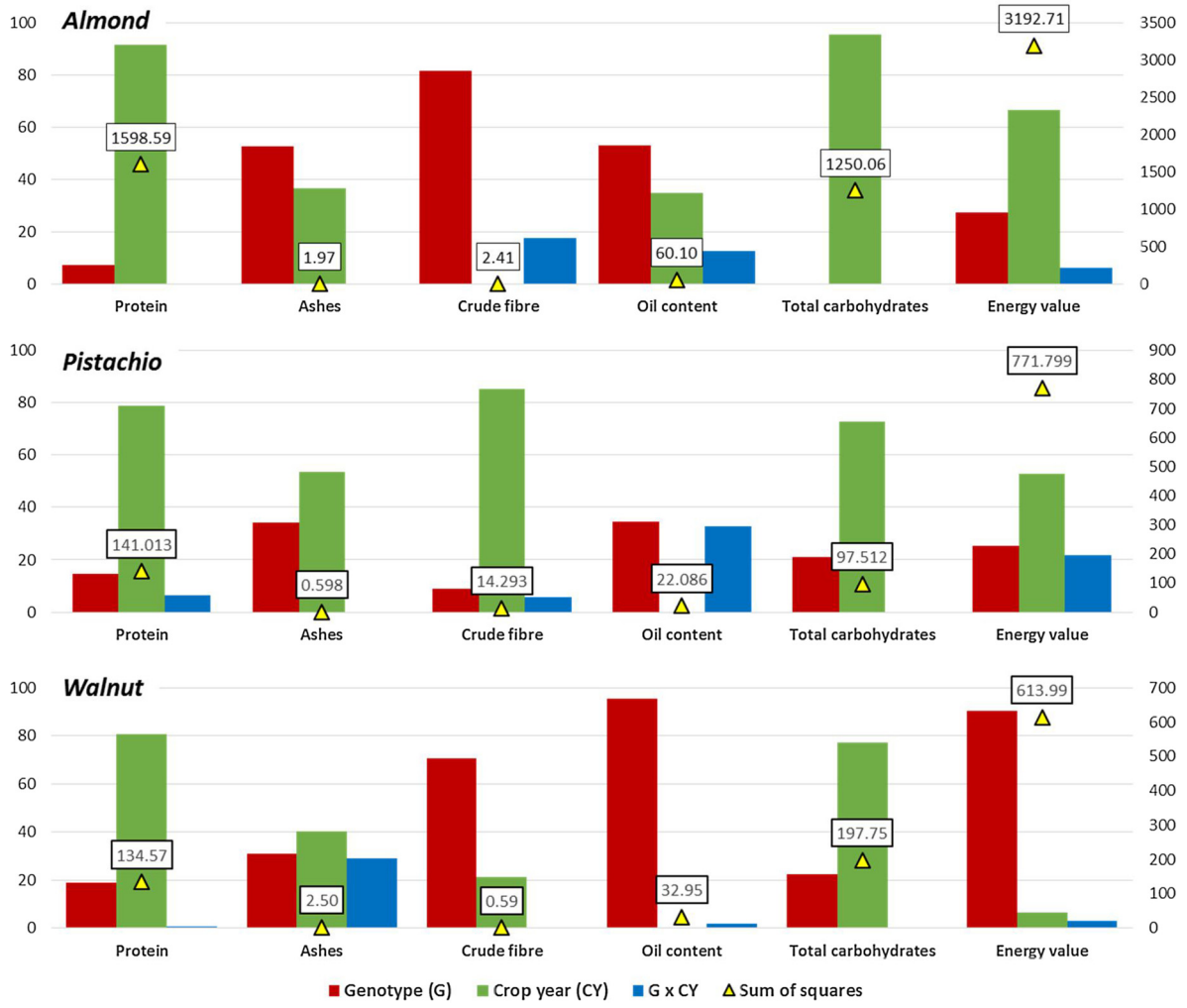

Fig. 2. Reported variability for the main nutritional components of nuts (almond, pistachio and walnut) components. Triangles (Δ) show the total variability of each chemical parameter by using the total sum of squares (right axis) after two-way ANOVA. Colour bars for each chemical parameter show the variability expressed as percentage (%) of the total sum of squares for genotype (G), crop year (CY) and the interaction of both (G x CY) (left axis).

mostly caused that variability is shown in Fig. 2. Within main nutritional components in nuts, protein concentration is the one with the highest reported variability, especially in almonds. In almonds and pistachios variability in the protein content was mainly caused by the CY (79 – 92%). Similarly, the content of carbohydrates was also mainly determined for the CY conditions in the three nuts considered. This reported variability in the content of proteins and carbohydrates in walnuts is in agreement with the study of Kodad et al. (2016 for Moroccan kernels.

The variability in the content of oil in nuts was lower than for previous components; however, it was higher in almonds than in walnuts and pistachios. The genotype was for the three nuts considered the main source of variability for this parameter. A larger effect of the genotype in the oil content of almonds and walnuts had been previously stated (Kodad et al., 2014, 2016). Differences in the cultivars considered and in the weather conditions on the crop site could be the cause of the reported differences in the results. Variability in the total content of ashes was reduced compared to previous components. The variability in the content of crude fibre was also reduced for almonds and walnuts, but not for pistachios.

One of the parameters greatly affected by genotype, CY and the interaction of both was the energy value of nuts. The strong influence of the genotype on the oil content of walnuts led to a higher effect of this factor on walnut nutritional value. The variability reported on the energy value in almonds was especially high, showing in this case a greater effect of the CY. As energy value is determined by calculation using nuts composition, the reported variability is the result of differences in the reported variability in the content of oil, proteins and carbohydrates.

Fig. 3 shows the results of the study of the variability observed in the fatty acid profile and minor components of nutritional interest in nuts. Previous studies show that by using different genotypes, almonds and pistachios with different fatty acids profiles can be produced (Mahmoodabadi et al., 2012; Roncero et al., 2016; Yada et al., 2011). In our study, the total variability observed in the fatty acid profile was in general small for almonds been higher in pistachios and walnuts. In almonds, the concentration of all specific fatty acids was significantly affected for the genotype in agreement with previous studies (García-López et al., 1996; Maestri et al., 2015). On the other hand, most of walnut fatty acids (linoleic, palmitic and palmitoleic) were similarly affected by the genotype and the CY. Previously a larger effect of the cultivar on walnut fatty acid profile had been described (Martínez et al., 2006). As previously mentioned, these differences as expected as they can be the result of the different cultivars considered and the variability in the yearly weather conditions. In pistachios, shorter chain fatty acids were mostly affected by the CY, while variability in the content of oleic and linoleic were almost exclusively determined by the genotype. The severe variability in the oleic and linoleic content of pistachio cultivars had been widely reported (Catalán et al., 2017; Mahmoodabadi et al., 2012; Seferoglu et al., 2006) and seems to overcome the effect of the

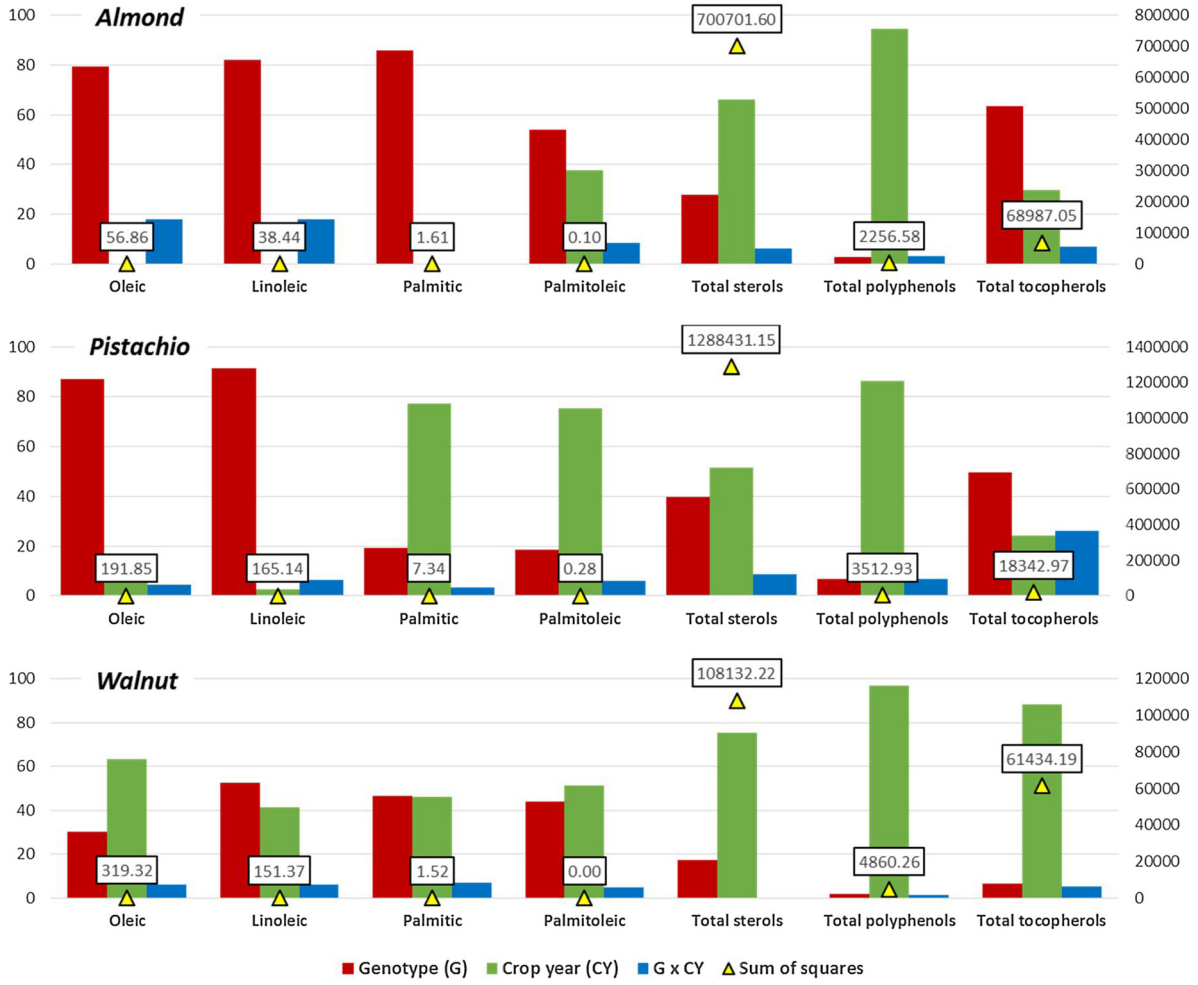

Fig. 3. Reported variability for the main nutritional components of nuts oils (almond, pistachio and walnut). Triangles (Δ) show the total variability of each chemical parameter by using the total sum of squares (right axis) after two-way ANOVA. Colour bars for each chemical parameter show the variability expressed as percentage of the total sum of squares for genotype (G), crop year (CY) and the interaction of both (G x CY) (left axis).

crop year.

The variability observed in the minor components concentration was particularly high. In the kernels considered, total phytosterols and especially the concentration of total polyphenols were highly affected by the CY. Specifically, phytosterols showed the greatest variations among all the considered nutritional components. Attending to specific factors, up to 55 – 75% of variability in the content of phytosterols can be directly attributed to CY conditions. In this regard, temperature and precipitation had been previously reported to have effect in the total phytosterols concentration in other species (Roche et al., 2010; Vlahakis and Hazebroek, 2000).

Other bioactive components that showed great variability were tocopherols. Although tocopherol content had been reported to be directly affected by temperature (Kodad et al., 2016), in our study the main source of variability in the content of tocopherols varied depending on the nut considered: the genotype was determinant for almonds and pistachios, while the CY was the most important factor in walnuts. In the case of walnuts, results for tocopherols agree with the larger effect of the cultivar found for a smaller number of cultivars in Argentina (Martínez et al., 2006). For almonds, similar results had been previously obtained for the content of α-tocopherol in cultivars grown in Argentina (Maestri et al., 2015) and local Spanish almond cultivars (Kodad et al., 2014).

Relevant concentrations of essential minerals were found on nuts in agreement with previous studies (Harmankaya et al., 2014; Segura et al., 2006). Specifically, five different macro minerals and five micro minerals appeared in significant concentrations. In this regard, the total variability reported for micro minerals was greater than for macro minerals (Fig. 4). The presence of minerals such as K, Ca, Mg, P, Fe, Zn, Mn and Cu improved the nutritional value of nuts as these minerals are an essential part of enzymes, and important play roles as catalysts and antioxidants (Gharibzahedi et al., 2012).

The highest variability appeared in the content of iron, manganese, zinc and copper. Analysing by factors, the variability reported in the content iron, manganese and copper was in the three nuts mainly determined by the CY. For the content of macro minerals, the results varied depending on the nut considered. A major effect of the genotype for macro minerals almonds and pistachios was reported, while in walnuts the CY seemed to be more determinant. Calcium possess inverse trend than others macrominerals with a mayor effect of the CY in pistachio and almond while in walnuts the genotype is more determinant. Attending to the results, the concentration crucial minerals such as iron, essential in blood formation (Gharibzahedi et al., 2014) or copper, involved in normal carbohydrate and lipid metabolism (Kabas et al., 2007), or zinc were mainly determined by the environmental conditions associated to the CY.

4. Conclusion

It should be considered that the crop years analysed only small variations on average annual temperatures and precipitation. However, the differences in the monthly distribution of temperatures and rainfall

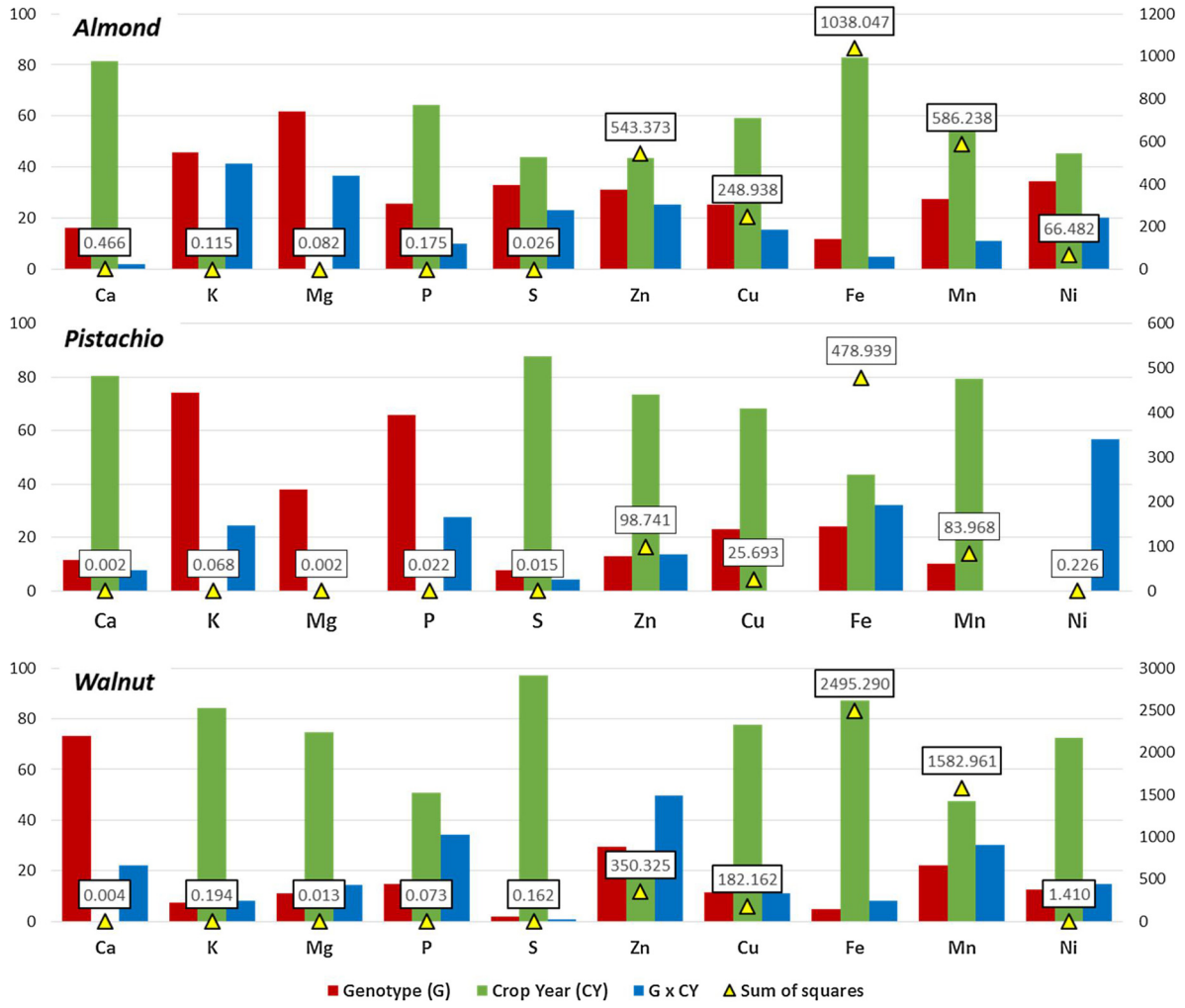

Fig. 4. Reported variability for the main essential minerals in nuts (almond, pistachio and walnut). Triangles (Δ) show the total variability of each mineral by using the total sum of squares (right axis) after two-way ANOVA. Colour bars for each mineral show the variability expressed as percentage of the total sum of squares for genotype (G), crop year (CY) and the interaction of both (G x CY) (left axis).

are enough to cause important effects on nuts nutritional components. These results confirm the importance of weather conditions on nuts health-promoting properties even when additional irrigation is used to meet crop water requirements. If crop production is not developed under completely controlled conditions (e.g. greenhouses) a significant variability of weather conditions on their chemical composition must be assumed. Therefore the predicted increase of extreme weather conditions due to global warming (Argüeso et al., 2016; Kam et al., 2016) is expected to have major effect on some of nuts health-promoting properties as protein content or concentration of bioactive compounds.

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